

Prediction of Dissolution-Absorption Relationships from a Continuous Dissolution/Caco-2 System

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ABSTRACT The objectives were 1) to design a continuous dissolution/Caco-2 system to predict the dissolution-absorption relationships for fast and slow dissolving formulations of piroxicam, metoprolol tartrate, and ranitidine HCl, and compare the predicted relationships with observed relationships from clinical studies; 2) to estimate the effect of croscarmellose sodium on ranitidine dissolution-absorption relationships; and 3) to estimate the effect of solubilizing agents on piroxicam dissolution-absorption relationships. A continuous dissolution/Caco-2 system was constructed from a dissolution apparatus and a diffusion cell, such that drug dissolution and permeation across a Caco-2 monolayer would occur sequentially and simultaneously. The continuous system generally matched observed dissolution-absorption relationships from clinical studies. For example, the system successfully predicted the slow metoprolol and slow ranitidine formulations to be permeation-rate-limited. The system predicted the slow piroxicam formulation to be dissolution-rate-limited, and the fast piroxicam formulation to be permeation-rate-limited, in spite of piroxicam's high permeability and low solubility. Additionally, the system indicated croscarmellose sodium enhanced ranitidine permeability and predicted solubilizing agents to not modulate permeability. These results suggest a dissolution/Caco-2 system to be an experimentally based tool that may predict dissolution-absorption relationships from oral solid dosage forms, and hence the relative contributions of dissolution and permeation to oral drug absorption kinetics.

INTRODUCTION

A dissolution/Caco-2 system was previously developed (1) to predict dissolution-absorption relationships of oral solid dosage forms prior to human studies. For the purposes of this work, this system is denoted the "two-step" dissolution/Caco-2 system. In the two-step system, dissolution samples

were first generated and treated with concentrated buffers to render them suitable (eg, isotonic) for Caco-2 monolayer permeation studies. In a second step, treated dissolution samples were then subjected to conventional Caco-2 flux studies. The two-step system yielded dissolution-absorption relationships that matched observed relationships from clinical studies. The two-step system represents an experimentally based approach to estimate the relative contributions of dissolution and permeation to overall absorption kinetics and does not rely on mathematical modeling approaches, which frequently impose model assumptions.

The scope of this work was to improve the two-step system by further integrating the dissolution and permeability components. A continuous dissolution/Caco-2 system is described here, where dissolution and permeation across Caco-2 monolayers occur simultaneously and sequentially. A Caco-2 monolayer is configured adjacent to dissolution media, into which a dosage form is dissolving.

The motivation for this work was the possibility that the two-step system may not detect excipient effects on drug permeability due to dissolution sample treatment. A laboratory tool that predicts dissolution-absorption relationships, including an excipient-mediated absorption influence, may be of value. A continuous dissolution/Caco-2 system was designed to be such a tool.

Three objectives were pursued. The first objective was to design and apply a continuous dissolution/Caco-2 system to predict dissolution-absorption relationships for fast and slow dissolving formulations of piroxicam, metoprolol tartrate, and ranitidine HCl, and compare the predicted relationships with observed relationships from clinical studies. Given the relative predictability of

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this continuous system and results from the ranitidine formulations, the second objective was to estimate the effect of croscarmellose sodium on ranitidine dissolution-absorption relationships. The third objective was to estimate the effect of solubilizing agents on piroxicam dissolution-absorption relationships.

MATERIALS AND METHODS

Fast and slow dissolving "immediate release" formulations of 20-mg piroxicam capsules, 100-mg metoprolol tartrate tablets, and 300-mg ranitidine HCl tablets were evaluated by the continuous dissolution/Caco-2 system. These formulations were previously developed at the University of Maryland School of Pharmacy and examined in crossover bioequivalence studies. These formulations and their clinical results have been reported (2-5). For simplicity, the fast and slow formulations of each drug are also denoted FAST and SLOW, respectively. Major differences between the fast and slow piroxicam formulations were that FAST employed micronized drug, sodium lauryl sulfate (SLS) as a dissolution aid, and lactose rather than microcrystalline cellulose as a filler (2). Major differences between the slow and fast metoprolol tartrate formulations were that FAST employed extra- rather than intragranular microcrystalline cellulose, a higher level of sodium starch glycolate, and a lower level of povidone (3). Major differences between the fast and slow ranitidine formulations were that FAST employed more lactose (less microcrystalline cellulose) as a filler and croscarmellose sodium as a disintegrant (4,5). Additional piroxicam formulations, which were not clinically tested, were also subjected to the dissolution/Caco-2 system and are described below. Piroxicam, metoprolol tartrate, and ranitidine HCl were USP grade. The FMC Corporation (Philadelphia, PA) provided croscarmellose sodium (Ac-Di-Sol). β -cyclodextrin was purchased from Roquette (Keokuk, IA).

The Continuous Dissolution/Caco-2 System

The continuous dissolution/Caco-2 system is illustrated in Figure 1 and consisted of a Van Kel dissolution apparatus (Cary, NC) and a side-by-side diffusion cell (Precision Instrument Design, Los Altos, CA). In this system, dissolution and

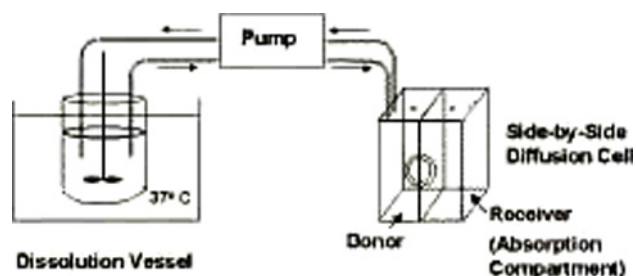


Figure 1. Schematic of the continuous dissolution/Caco-2 system.

permeation across Caco-2 cells occurred simultaneously. As drug dissolved, dissolution medium was filtered through a 10- μ m dissolution filter and transferred via a peristaltic pump (Rainin Instruments Inc, Woburn, MA) to the donor compartment of the side-by-side diffusion cell. Mounted between the donor and receiver compartments was a Caco-2 monolayer grown on a polycarbonate Snapwell filter. Dissolved drug was available to permeate into the receiver (or absorption) compartment. Dissolution medium recirculated from the donor compartment, back to the dissolution vessel. Polyvinylchloride tubing (internal diameter = 1.59 mm) connected the dissolution vessel and diffusion cell. The flow rate was 8.3 mL/min.

Caco-2 cells were grown in T-75 flasks at 37°C in an atmosphere of 5% CO₂ and 95% RH using Delbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acids and 0.05% penicillin/streptomycin. Medium was changed approximately every 48 hours. Cells were passaged at 80-90% confluency using a 0.20% EDTA / 0.25% trypsin solution. Cells from passages 35-55 were seeded onto polycarbonate Snapwell filters (0.4 μ m pore size; 1.0 cm² area) at a density of 4 x 10⁵ cells/cm². Cells were cultured for 21 to 28 days and subsequently inserted into the side-by-side diffusion cell of the dissolution/Caco-2 system.

Dosage Form Evaluation in the Continuous Dissolution/Caco-2 System

Each formulation was evaluated by the dissolution/Caco-2 system at 37°C. The dissolution component of the continuous dissolution/Caco-2 system was performed using compendial methods

(6), except for changes to the content and volume of the dissolution medium. Hank's Balanced Salts Solution (HBSS) at pH 6.8 was used as the dissolution medium, in order to accommodate the Caco-2 monolayer. HBSS is an isotonic solution that supports the viability of living cells. The compendial dissolution medium for piroxicam and metoprolol tartrate was simulated gastric fluid (SGF), and for ranitidine HCl, was deionized water. SGF and deionized water do not support Caco-2 cell viability.

Dissolution volumes for the piroxicam, metoprolol, and ranitidine formulations were 500, 300, and 250 mL, respectively. For all three drugs, compendial methods use volumes of 900 mL. The rationale for reduced volumes is to mimic the chyme volume in the gastrointestinal tract, while maintaining sink conditions. Dressman et al. suggested 250 mL (7). The solubility of piroxicam, metoprolol tartrate, and ranitidine HCl in HBSS allowed for dissolution sink conditions (1).

For the fast dissolving formulations, dissolution samples were taken with replacement at 5, 10, 15, 20, 30, 40, 50, and 60 minutes. For the slow dissolving formulations, dissolution samples were taken with replacement at 15, 30, 45, 60, 75, 90, 105, and 120 minutes. Samples from the dissolution vessels were filtered through a 0.2 μm filter and analyzed by HPLC.

The permeability component of the dissolution/Caco-2 system consisted of the side-by-side diffusion chambers. The receiver (or absorption) compartment was 7 mL of HBSS. Sink conditions across the Caco-2 monolayer persisted throughout all studies. For the fast dissolving formulations, the absorption compartment was sampled with replacement at 8, 13, 18, 23, 33, 43, 53, and 63 minutes. For the slow dissolving formulations, the absorption compartment was sampled with replacement at 18, 33, 48, 63, 78, 93, 108, and 123 minutes. To allow time for drug to circulate from the dissolution vessel to the Caco-2 monolayer surface, absorption samples were collected three minutes later than their corresponding dissolution samples. Samples were analyzed by HPLC.

Drug permeability across the Caco-2 monolayer was calculated using

$$P_{\text{eff}} = (dM/dt)/AC \quad (1)$$

where P_{eff} is effective drug permeability, dM is the drug accumulation in the absorption compartment during time interval dt , A is the surface area, and C is drug concentration in the donor compartment. Monolayer integrity was evaluated by transepithelial electrical resistance (TEER) in HBSS throughout the course of the study ($> 650\Omega \text{ cm}^2$ at 37°C , after subtracting a "filter" resistance of $150\Omega \text{ cm}^2$) and by parallel mannitol permeability studies. Mass balance ranged between 90% and 105% for all continuous dissolution/Caco-2 studies.

Predicted Dissolution-Absorption Relationships from the Continuous Dissolution/Caco-2 System and Comparison With In Vivo Data

For each fast and slow formulation of piroxicam, metoprolol, and ranitidine, dissolution-absorption relationships were constructed by plotting the fraction drug absorbed (F_a) against the fraction drug dissolved (F_d) at identical times. The ability of this system to predict observed in vitro dissolution-in vivo absorption profiles in humans was assessed by comparing predicted relationships from the dissolution/Caco-2 system with those obtained from clinical studies. For simplicity, the manuscript refers to three categories of dissolution-absorption plots: "straight line," "reverse L," and "hockey stick." A "straight line" relationship between F_a and F_d indicates dissolution-rate-limited absorption; a "reverse L" appearing profile indicates permeation-rate-limiting absorption; an intermediate "hockey stick" profile indicates both dissolution and permeation contribute nearly equally to overall drug absorption kinetics (8). These categories were previously invoked from idealized considerations of absorption-dissolution phase plane plots (8). Clearly, such categorizations here are simplifications of drug absorption phenomena and kinetics.

It should be noted that while HBSS was the dissolution medium in the continuous dissolution/Caco-2 system, compendial dissolution results were applied in the generation of the previously reported clinical F_a vs F_d profiles (9,10). Since dissolution was similar in compendial media and HBSS for all formulations (data not shown), this use of differing media for the dissolution/Caco-2 and clinical trajectories was of no significance. Since

dissolution in HBSS was similar to dissolution in the compendial test, dissolution in HBSS and compendial medium were assumed to represent in vivo dissolution.

A primary benefit of this dissolution/Caco-2 system is that it is experimentally based and does not employ mathematical models, which typically require simplifying assumptions. However, so that its predictability could be more readily assessed, the F_a vs F_d profiles from the continuous system were fit to

$$F_a = f_a^{-1} [1 - \alpha(\alpha - 1)^{-1}(1 - F_d) + (\alpha - 1)^{-1}(1 - F_d)^\alpha] \quad (2)$$

where F_a is the fraction of the total amount of drug absorbed at time t , t_a is the fraction of the dose absorbed at $t = \infty$, α is the ratio of the apparent first-order permeation rate constant to the first-order dissolution rate constant, and F_d is the fraction of the dose dissolved at time t . Assumptions of this model have been previously described (8). For all formulations, α was determined from Eq. 2 and compared to previously observed α values from clinical studies (9,10).

Effect of Disintegrating Agent on Ranitidine Dissolution-Absorption Relationships

A second objective was to estimate the effect of croscarmellose sodium on ranitidine dissolution-absorption relationships. The fast ranitidine formulation was subjected to dissolution/Caco-2 system evaluation with 0 mg, 35 mg, and 65 mg of croscarmellose sodium added to the HBSS dissolution medium. These three test conditions are denoted the formulation FAST, "FAST plus 35 mg croscarmellose," and "FAST plus 65 mg croscarmellose," respectively.

As the results show, croscarmellose enhanced ranitidine permeability across the Caco-2 monolayer in the dissolution/Caco-2 systems. Permeability-only studies of ranitidine across Snapwell filters were conducted in HBSS containing 0, 0.001, 0.01, and 0.06 mg/mL croscarmellose sodium. Mass balance ranged from 90% to 100%.

Effect of Solubilizing Agent on Piroxicam Dissolution-Absorption Relationships

A third objective was to estimate the effect of a solubilizing agent on piroxicam dissolution-absorption relationships. Two variant formulations of piroxicam FAST were fabricated, one where 1% w/w Tween-80 was substituted for the 1% wt/wt SLS (denoted "FAST with Tween") and one where SLS was deleted (denoted "FAST without SLS"). "FAST with Tween" and "FAST without SLS" were manufactured as FAST had been (2), except the two variant formulations were hand-filled into capsules. "FAST with Tween" and "FAST without SLS" were subjected to the continuous dissolution/Caco-2 system, as was FAST.

Additionally, two variant formulations of piroxicam SLOW were fabricated, one denoted "SLOW with β -cyclodextrin" and one denoted "SLOW with SLS." "SLOW with β -cyclodextrin" contained 213.5 mg of β -cyclodextrin, in place of the microcrystalline cellulose in SLOW. "SLOW with SLS" contained 2% wt/wt of SLS (5 mg), in place of some microcrystalline cellulose in SLOW. "SLOW with β -cyclodextrin" and "SLOW with SLS" were manufactured as SLOW had been (2), except the two variant formulations were hand-filled into capsules. "SLOW with β -cyclodextrin" and "SLOW with SLS" were subjected to the continuous dissolution/Caco-2 system, as was SLOW.

Since the variant formulations were not clinically evaluated but employed modest changes in the solubilizing agent, it was anticipated that changes in the dissolution-absorption relationship could result from dissolution changes, but not permeability changes. In this regard, these studies examining the effect of modest levels of solubilizing agent on piroxicam F_a vs F_d profiles represent negative control studies.

RESULTS AND DISCUSSION

Predicted Dissolution-Absorption Relationships from the Continuous Dissolution/Caco-2 System and Comparison With In Vivo Data: Piroxicam

Predicted dissolution-absorption relationships from the continuous dissolution/Caco-2 system for the piroxicam fast and slow formulations are plotted in Figures 2 and 3, respectively. The predicted relationship for FAST exhibited a "reverse L"

appearance, characteristic of overall drug absorption that is permeation-rate-limited. The predicted relationship for SLOW yielded a "hockey stick" appearance, characteristic of mixed dissolution and intestinal permeation-rate-limited absorption. Hence, the continuous system predicted that dissolution is nearly complete from FAST before appreciable absorption takes place, while dissolution and permeation are about equally rate limiting for SLOW.

Also plotted in Figures 2 and 3 are the observed F_a vs F_d profiles from clinical studies for FAST and SLOW, respectively (10). For FAST, the predicted and observed profiles match and indicate permeation-rate-limiting absorption. For SLOW, the predicted profile did not quantitatively match the observed relationship. The predicted relationship overestimated the observed degree of permeation-rate-limited absorption. While the observed profile had a "straight line" appearance, which suggests marked dissolution-rate-limited absorption, the predicted relationship gave a "hockey stick" appearance (ie mixed dissolution and permeation-rate-limited absorption).

In addition to evaluating the predictive capability of the continuous system by comparing dissolution-absorption relationships, this system was also evaluated by comparing a values from eq 2 of the F_a vs F_d profiles. α is a dimensionless parameter reflecting the degree to which dissolution limits overall drug absorption kinetics. An α value much greater than 1.0 indicates dissolution-rate-limited absorption. An α value much less than 1.0 indicates permeation-rate-limited absorption. An α value of 1.0 indicates perfectly mixed dissolution- and permeation-rate-limited absorption.

Table 1 lists α values from both the continuous system (predicted) and clinical studies (observed). For piroxicam, the continuous system predicted permeation-rate-limited absorption for FAST ($\alpha = 0.195$) and mostly dissolution-rate-limited absorption for SLOW ($\alpha = 1.18$). From the perspective of oral solid dosage form biopharmaceutic characterization, these predictions generally agree with the observed relationships of FAST ($\alpha = 0.896$) and SLOW ($\alpha = 6.50$). The dissolution/Caco-2 system correctly classified

FAST and SLOW as permeation-controlled (ie $\alpha < 1$) and dissolution-controlled (ie $\alpha > 1$), respectively. However, in comparing α values, it is evident that predicted relationships were about five-fold more permeation-rate-limited than the observed relationships for both FAST and SLOW.

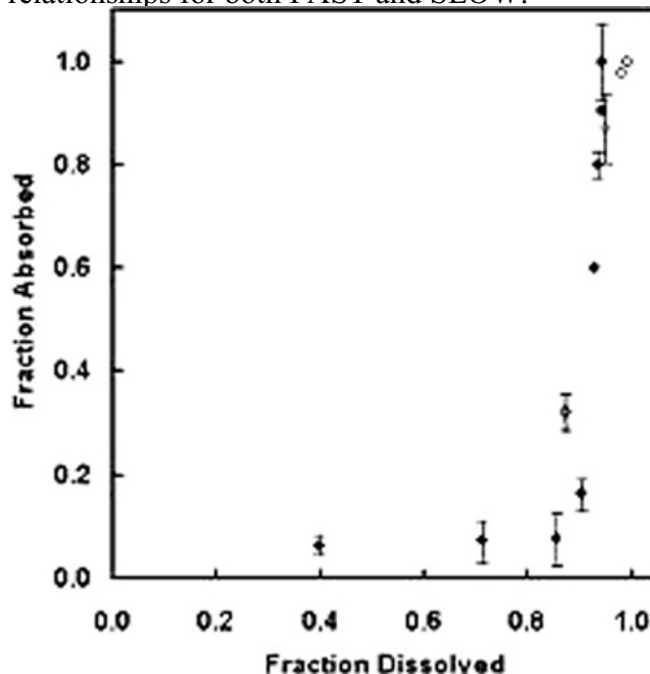


Figure 2. Predicted and observed F_a vs F_d relationships of fast piroxicam formulation. The predicted relationship (closed symbol) from the continuous dissolution/Caco-2 system was similar to the observed relationship (open symbol). Each exhibited a "reverse L" profile, characteristic of permeation-rate-limited absorption.

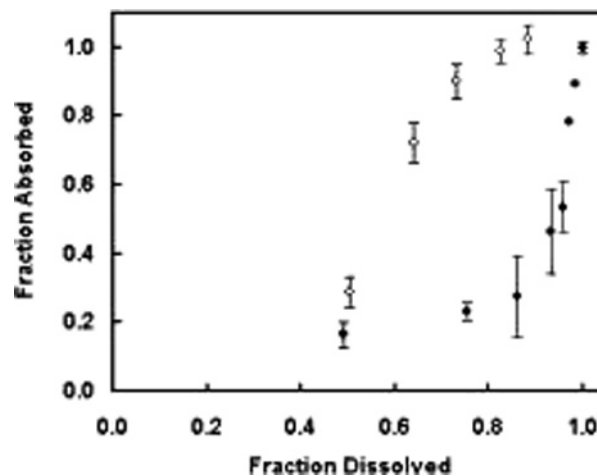
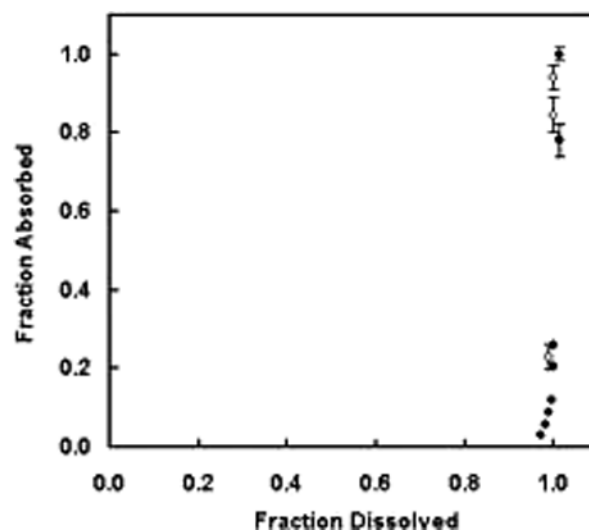
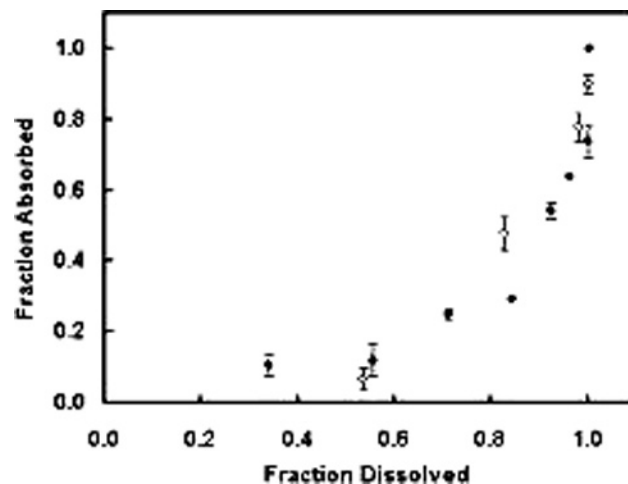


Figure 3. Predicted and observed F_a vs F_d relationships of slow piroxicam formulation. The predicted relationship (closed symbol) from the continuous dissolution/Caco-2 system overestimated the degree of permeation-rate-limited absorption from the observed relationship (open symbol). This discrepancy may be due to a lower Caco-2 permeability, relative to human intestinal permeability.

Table 1. Comparison of Predicted Dissolution-Absorption Relationships from a Continuous Dissolution/Caco-2 System With Observed Relationships

Drug	Formulation	Observed α	Predicted α
Piroxicam	FAST	0.896 (± 0.138)	0.195 (± 0.037)
Piroxicam	SLOW	6.50 (± 2.17)	1.18 (± 0.24)
Metoprolol tartrate	FAST	0.0743 (± 0.0178)	0.00648 (± 0.00049)
Metoprolol tartrate	SLOW	0.648 (± 0.103)	0.415 (± 0.072)
Ranitidine	FAST	0.0646 (± 0.010)	0.454 (± 0.115)
Ranitidine	SLOW	0.156 (± 0.020)	0.130 (± 0.006)

In general, for highly permeable drugs (11,12), and for piroxicam (10,13), Caco-2 monolayers are several-fold less permeable than human jejunum, which is consistent with the above results. We have previously measured piroxicam's Caco-2 permeability to be $0.913 (\pm 0.012) \times 10^{-4}$ cm/sec (10). Piroxicam's human jejunal permeability was measured to be $10.40 (\pm 5.40) \times 10^{-4}$ cm/sec (13), about 10-fold higher. These permeability determinations suggest that the predicted relationships of piroxicam here, in Figures 2 and 3, were several-fold more permeation-rate limiting than the observed relationships due to a several-fold lower permeability across the Caco-2 monolayer relative to human jejunum. The differences between the predicted and observed dissolution-absorption relationships may be attributed to intrinsic differences between in vivo and in vitro permeability, particularly for piroxicam SLOW in Figure 3. As shown in Figure 1 of reference 8, the trajectory of the dissolution-absorption phase plane profile is most sensitive to α , in the neighborhood of $\alpha = 1$, as is the case for piroxicam SLOW. Hence, while the predicted and observed relationships in Figure 3 are quantitatively different, the dissolution/Caco-2 system correctly classified SLOW as dissolution-controlled (ie $\alpha > 1$), in contrast to FAST. To obtain greater quantitative agreement, the permeability component to the present system needs improved accuracy (ie made more permeable).

Figure 4. Predicted and observed F_a vs F_d relationships of fast metoprolol tartrate formulation. The predicted relationship (closed symbol) from the continuous dissolution/Caco-2 system was similar to the observed relationship (open symbol).Figure 5. Predicted and observed F_a vs F_d relationships of slow metoprolol tartrate formulation. The predicted relationship (closed symbol) from the continuous dissolution/Caco-2 system was similar to the observed relationship (open symbol).

In the Biopharmaceutical Classification System (BCS), piroxicam is a "high permeable, low soluble" drug (Class II), and suggests piroxicam may be dissolution-rate-limited. However, the continuous system indicated that the piroxicam fast formulation was permeation-rate-limited, in agreement with the observed clinical data. Major differences between the fast and slow piroxicam formulations were that FAST employed micronized drug, SLS as a dissolution aid, and lactose rather than microcrystalline cellulose as a filler (2). Like

the two-step dissolution/Caco-2 system (1), the continuous system predicted the effect of these "favorable" formulation changes from slow formulation to fast formulation, would be that FAST was permeation-rate-limited. Hence, this continuous system was able to predict the relative contributions of dissolution and permeation to overall drug absorption kinetics for fast piroxicam, in spite of piroxicam's high permeability and low solubility.

Predicted Dissolution-Absorption Relationships from the Continuous Dissolution/Caco-2 System and Comparison With In Vivo Data: Metoprolol Tartrate

The predicted dissolution-absorption relationships for fast and slow dissolving formulations of metoprolol are plotted in Figures 4 and 5, respectively. The predicted relationships of both formulations exhibited a "reverse L" appearance, characteristic of permeation-rate-limited drug absorption. For both formulations, the continuous system predicted nearly complete dissolution prior to appreciable absorption, with FAST more permeation-rate-limited than SLOW. Also plotted in Figures 4 and 5 are the observed relationships from clinical studies (10). There was general agreement between the observed profiles and those predicted from the continuous system. For both FAST and SLOW, the predicted and observed relationships exhibited a "reverse L" appearance.

The predicted relationships were also evaluated by comparing their α values with observed α values from clinical studies. In Table 1, the continuous system predicted permeation-rate-limited absorption from FAST ($\alpha = 0.00648$) and SLOW ($\alpha = 0.415$) for metoprolol. These predictions are similar to FAST ($\alpha = 0.0743$) and SLOW ($\alpha = 0.648$) from clinical studies. It should be noted that large relative differences in fitted α values do not necessarily indicate practically meaningful differences in kinetic interpretation. In Figure 1 of reference 8, the trajectory of the dissolution-absorption phase plane profile shows low sensitivity to α , in the neighborhood of $\alpha = 0.01$, as is the case for metoprolol FAST. Hence, although the predicted and observed α values for FAST differ 10-fold, each indicates marked permeation-rate-limited

absorption. In Figure 4 for metoprolol FAST, the predicted and observed profiles are clearly very similar; the timing of the first data point along the trajectory mostly determined the fitted α value for small α .

In the BCS, metoprolol tartrate is a "high permeable, high soluble" drug (Class I). From its solubility and permeability, it would be difficult to predict whether overall absorption is dissolution- or permeation-rate-limited, since metoprolol tartrate both dissolves and permeates rapidly. Like the two-step dissolution/Caco-2 system (1), this continuous system predicted permeation-rate-limited metoprolol absorption from both formulations, which agreed with clinical observations.

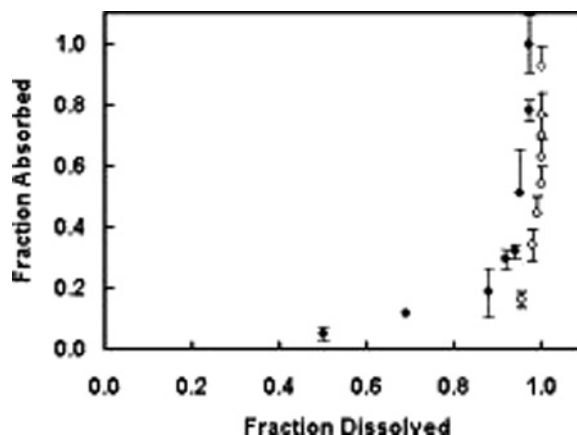


Figure 6. Predicted and observed F_a vs F_d relationships of fast ranitidine HCl formulation. The predicted relationship (closed symbol) from the continuous dissolution/Caco-2 system was similar to observed relationship (open symbol).

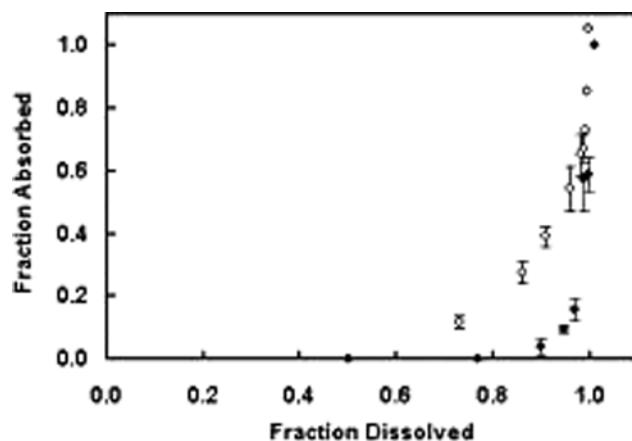


Figure 7. Predicted and observed F_a vs F_d relationships of slow ranitidine HCl formulation. The predicted relationship (closed symbol) from the continuous dissolution/Caco-2 system was similar to the observed relationship (open symbol).

Table 2. Ranitidine Permeability across Caco-2 Monolayers in Continuous Dissolution/Caco-2 System

Formulation	$P_{\text{eff}} \times 10^6$ (cm/sec)
SLOW ^a	0.230 (⁺ 0.023)
FAST ^b	0.655 (⁺ 0.217)
"FAST with 35 mg croscarmellose"	1.56 (⁺ 0.59)
"FAST with 65 mg croscarmellose"	2.34 (⁺ 0.76)

^aSLOW contained no croscarmellose sodium.^bFAST contained 15 mg croscarmellose sodium per tablet, yielding 0.06 mg/mL of croscarmellose sodium after tablet disintegration.

Predicted Dissolution-Absorption Relationships from the Continuous Dissolution/Caco-2 System and Comparison With In Vivo Data : Ranitidine HCl

The predicted dissolution-absorption relationships for the fast and slow dissolving ranitidine formulations are plotted in Figures 6 and 7, respectively, along with their observed relationships (10). There is general agreement between the predicted and observed profiles. All profiles exhibited a "reverse L" appearance, characteristic of permeation-rate-limiting ranitidine absorption. The continuous system predicted nearly complete dissolution before appreciable absorption. In Table 1, the continuous system predicted permeation-rate-limited absorption from FAST ($\alpha = 0.454$) and SLOW ($\alpha = 0.130$). These predictions were similar to the observed relationships of FAST ($\alpha = 0.0646$) and SLOW ($\alpha = 0.156$). Like the two-step dissolution/Caco-2 system (1), the continuous system generally agreed with the observed relationships and indicated permeation-rate-limited ranitidine absorption from both formulations.

In spite of the general agreement between predicted and observed trajectories, the F_a vs F_d trajectory for fast ranitidine formulation was more dissolution-rate-limited than for slow ranitidine formulation, in disagreement with the observed results and results from the two-step dissolution/Caco-2 system. The predicted α value for FAST was seven-fold larger than the observed FAST value, and even three-fold higher than the predicted SLOW value. The explanation for this high prediction for FAST was

an increased permeability across the Caco-2 monolayer for FAST, which utilized 15 mg of croscarmellose sodium per tablet as a super-disintegrant (4,5). In Table 2, P_{eff} from continuous system studies of slow and fast formulations were 0.230×10^{-6} cm/sec and 0.655×10^{-6} cm/sec, respectively. P_{eff} was three-fold higher from the fast formulation, such that FAST was three-fold more permeation-rate-limited than SLOW. Additional results below show croscarmellose enhanced ranitidine permeability in further dissolution/Caco-2 system experiments and in conventional Caco-2 monolayer permeability studies.

It should be noted that while this continuous system correctly predicted fast and slow ranitidine formulations to be permeation-rate-limited, the observed in vivo ranitidine absorption rate was the same for FAST and SLOW (11). Hence, from an in vivo interpretation standpoint, the continuous system was overly sensitive (ie false positive) to a croscarmellose permeation-enhancing effect. The two-step system did not show a false positive effect (1), perhaps due to sample dilution in the two-step system protocol.

In the BCS, ranitidine HCl is considered to be a "low permeable, high soluble" drug (Class III). This classification correctly suggests that absorption is permeation-rate-limited. Like the two-step system, this continuous system simply agreed with this correct prediction from discrete solubility and permeability studies.

In summary for the clinically evaluated fast and slow formulations of piroxicam, metoprolol, and ranitidine, predicted dissolution-absorption relationships from a continuous dissolution/Caco-2 system qualitatively matched in vivo data, and frequently quantitatively matched in vivo data. Except for fast ranitidine, the predicted relationships slightly overestimated the degree of permeation-rate-limited absorption (ie underpredicted the α values), perhaps due to the lower Caco-2 permeability relative to human intestine. This effect, in the neighborhood of $\alpha = 1$ where the dissolution-absorption relationship is most sensitive to changes in α , resulted in a qualitative but not quantitative agreement between predicted and observed profiles for piroxicam

SLOW (Figure 3). Unlike discrete solubility and permeability data, the dissolution/Caco-2 systems correctly predicted the relative contributions of dissolution and permeability to absorption from the fast piroxicam formulation and from the metoprolol fast and slow formulations. Results from this continuous dissolution/Caco-2 system matched results from the previously reported, simpler, two-step dissolution/Caco-2 system.

Effect of Disintegrant on Ranitidine Dissolution-Absorption Relationships

Given the predictability of this continuous system, the system was employed to estimate excipient effects on dissolution and permeation, and the resulting dissolution-absorption profile. Specifically, a second objective was to estimate the effect of the disintegrant croscarmellose sodium on ranitidine dissolution-absorption relationships. These studies were motivated by the above observation where fast ranitidine formulation was more dissolution-rate-limited than slow ranitidine formulation, due to a three-fold increase in P_{eff} for FAST relative to P_{eff} for SLOW.

The F_a vs F_d trajectories from the continuous dissolution/Caco-2 system of SLOW, FAST, "FAST plus 35 mg croscarmellose", and "FAST plus 65 mg croscarmellose" are drawn in Figure 8. As noted above, addition of croscarmellose through a formulation change from SLOW (no croscarmellose) to FAST (with 15 mg croscarmellose) resulted in less permeation control of absorption (ie more dissolution control of absorption), in spite of FAST's more rapid dissolution. This influence of croscarmellose was further seen for "FAST plus 35 mg croscarmellose" and "FAST plus 65 mg croscarmellose". In fact, "FAST plus 65 mg croscarmellose" exhibited a "hockey stick" F_a vs F_d profile, which suggests mixed rate-limited absorption. Fitted α values for "FAST plus 35 mg croscarmellose" and "FAST plus 65 mg croscarmellose" were 0.630 (\pm 0.031) and 1.22 (\pm 0.08), respectively.

These profiles for FAST, "FAST plus 35 mg croscarmellose", and "FAST plus 65 mg croscarmellose" can be explained by a permeation-enhancing effect on ranitidine by croscarmellose. While dissolution in the continuous system was the

same for the three (data not shown), P_{eff} increased from 0.230×10^{-6} cm/sec for SLOW to 0.655×10^{-6} cm/sec for FAST, to 1.56×10^{-6} cm/sec for "FAST plus 35 mg croscarmellose", and to 2.34×10^{-6} cm/sec for "FAST plus 65 mg croscarmellose" (Table 2). The 10-fold increase in permeability from SLOW to "FAST plus 65 mg croscarmellose" resulted in "FAST plus 65 mg croscarmellose" being mixed dissolution and permeation-rate-limited ($\alpha = 1.22$), in spite of even SLOW being very permeation-rate-limited ($\alpha = 0.130$) and in spite of ranitidine's normally low permeability and high solubility (10).

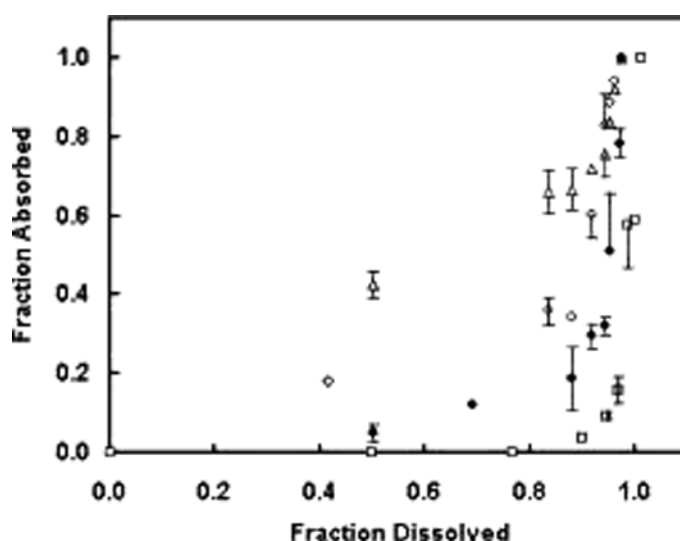


Figure 8. Predicted F_a vs F_d relationships of ranitidine SLOW (open square), ranitidine FAST (closed symbol), "FAST with 35 mg croscarmellose" (open symbol), and "FAST with 65 mg croscarmellose" (open triangle). The fast ranitidine formulation contained 15 mg croscarmellose sodium. Croscarmellose enhanced ranitidine permeability, such that the F_a vs F_d profiles became increasingly dissolution-rate-limited.

Table 3. Effect of Croscarmellose Sodium Concentration on Ranitidine Permeability across Caco-2 Monolayers

Croscarmellose Sodium Concentration (mg/mL)	$P_{\text{eff}} \times 10^6$ (cm/sec)
0	0.425 (\pm 0.058)
0.001	0.650 (\pm 0.032)
0.01	0.808 (\pm 0.085)
0.06	11.2 (\pm 0.1)

Conventional Caco-2 permeability-only studies of ranitidine confirm the permeation-enhancing properties of croscarmellose. In Table 3, ranitidine P_{eff} in HBSS containing 0, 0.001, 0.01, and 0.06 mg/mL croscarmellose sodium were 0.425×10^{-6} , 0.650×10^{-6} , 0.808×10^{-6} , and 11.2×10^{-6} cm/sec, respectively. Croscarmellose increase permeability over 20-fold (ANOVA P-value < 0.01).

The enhanced ranitidine permeability may be explained by compromised Caco-2 tight junctions, due to croscarmellose chelation of Ca^{2+} . Gan et al. (14) observed a 15- to 20-fold increase in ranitidine permeability when Ca^{2+} -free media or EDTA-containing media was used. Extracellular Ca^{2+} depletion causes disassembly of tight junctions and adherens junctions (15). For adherens junctions, Ca^{2+} is required for homotypic cadherin interactions. For tight junctions, intracellular consequences of reduced extracellular Ca^{2+} are complex and incompletely elucidated. However, the dependence of tight junction formation on intracellular Ca^{2+} is consistent with classic signaling pathways that utilize heterotrimeric G proteins and protein kinase C (PKC) (16).

Croscarmellose sodium is a cross-linked polymer of carboxymethylcellulose sodium, with a degree of substitution between 0.60 and 0.85. Croscarmellose binds cations in vitro (17), although this interaction has not always been of pharmaceutical consequence in vivo (18). For a degree of substitution of 0.7, 1 mmol of Ca^{2+} can be sequestered by 691.9 mg of croscarmellose sodium. FAST, "FAST plus 35 mg croscarmellose", and "FAST plus 65 mg croscarmellose" each may bind 0.022 mmol, 0.072 mmol, and 0.116 mmol Ca^{2+} , respectively. Since HBSS contains 0.00126 mM Ca^{2+} , 0.000324 mmol of Ca^{2+} resided in the dissolution/Caco-2 system. Hence, croscarmellose potentially can sequester all the system's Ca^{2+} . Tight junction disassociation due to Ca^{2+} sequestration by croscarmellose appears to have been the mechanism of enhanced ranitidine permeation in vitro, resulting in the switch from permeation-rate-limited absorption to dissolution-rate-limited absorption. Since ranitidine intestinal permeation kinetics from FAST and SLOW were the same in vivo (10), this continuous dissolution/Caco-2 system gave a "false positive" for croscarmellose's permeation-enhancing effect.

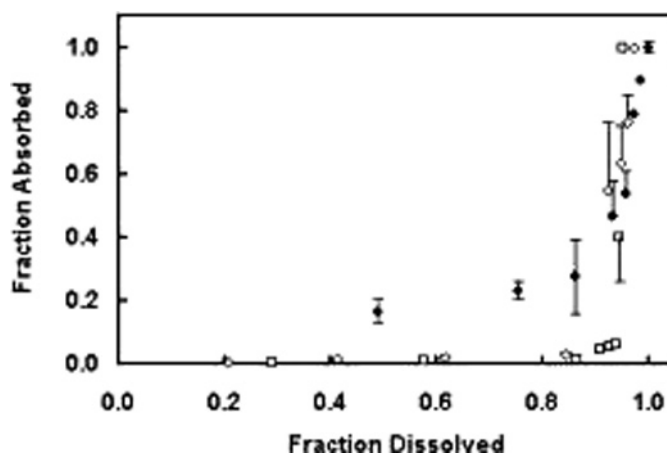


Figure 9. Predicted F_a vs F_d relationships of piroxicam SLOW (closed symbol), "SLOW with b-cyclodextrin" (open diamond), and "SLOW with SLS" (open square). The continuous dissolution/Caco-2 system predicted less dissolution control for "SLOW with b-cyclodextrin" and "SLOW with SLS" due to increased dissolution, with no excipient-mediated permeation enhancement.

Effect of Solubilizing Agent on Piroxicam Dissolution-Absorption Relationships

A third objective was to estimate the effect of common levels of solubilizing agents on the relative contributions of piroxicam dissolution and intestinal permeation, and to serve as a negative control study. Given the "false positive" results above for croscarmellose sodium, a continuous dissolution/Caco-2 system can only have value if it accurately predicts a lack of excipient effect on permeability. Solubilizing agents such as SLS and cyclodextrins possess dissolution-enhancing properties at modest, pharmaceutically-relevant concentrations (19-21). SLS and cyclodextrins have also shown permeation-enhancing properties (22-24), although may require higher concentrations provided by typical solid oral dosage formulations.

Two sets of experiments were performed. In one set, the piroxicam FAST and its two variants ("FAST with Tween" and "FAST without SLS") were evaluated. In the other set, the piroxicam SLOW and its two variants ("SLOW with β -cyclodextrin" and "SLOW with SLS") were evaluated. Solubilizing agent levels in these formulations were typical for capsules.

Dissolution of fast piroxicam and its two variant formulations were similar, although rank order was: FAST > "FAST with Tween" > "FAST without SLS". Dissolution-absorption relationships for FAST, "FAST with Tween", and "FAST without SLS" were permeation-rate-limited, and about equally so (data not shown). Piroxicam P_{eff} did not vary across formulations. Hence, the continuous system predicted minimal or no change in dissolution and permeation.

In the other set of experiments, added surfactants to the piroxicam SLOW increased dissolution rate five-fold; rank order was "SLOW with SLS" > "SLOW with β -cyclodextrin" > SLOW. Piroxicam P_{eff} did not vary across these formulations. Dissolution-absorption relationships for "SLOW with SLS", "SLOW with β -cyclodextrin", and SLOW are illustrated in Figure 9 and indicate markedly less dissolution control for "SLOW with β -cyclodextrin" and "SLOW with SLS", which was completely permeation-controlled. Fitted α values for SLOW, "SLOW with β -cyclodextrin", and "SLOW with SLS" were 1.18 (\pm 0.24), 0.552 (\pm 0.022), and 0.216 (\pm 0.028), respectively. Hence, the continuous system predicted an excipient effect of dissolution-absorption relationships due to dissolution, and not due to permeation.

Although these results were anticipated, these two sets of experiments support the utility of the dissolution/Caco-2 system as a tool to study excipient effects on dissolution and intestinal permeation kinetics. These results represent negative control studies for the effect of excipient on drug permeation, where excipient effect on dissolution but not permeation was anticipated. While even the most potent cyclodextrins, the methylated β -cyclodextrins, require concentrations of 20-50 mg/mL for permeation enhancement (24), "SLOW with β -cyclodextrin" yielded only a 0.421 mg/mL β -cyclodextrin concentration in the dissolution/Caco-2 system. A 0.115 mg/mL SLS can enhance permeability (22); however, piroxicam FAST and "SLOW with SLS" yielded only 0.005 and 0.01 mg/mL of SLS, respectively. A continuous dissolution/Caco-2 system can only have value if it accurately predicts a lack of excipient effect on permeability, as was the case here for β -

cyclodextrin and SLS. Such experience will help provide a basis to consider a permeation-enhancing effect of an excipient in future applications of a dissolution/Caco-2 system.

CONCLUSIONS

A two-step dissolution/Caco-2 system was previously shown to predict dissolution-absorption relationships and is a potential tool for characterizing prototype formulations and setting dissolution specifications (9). The scope of this work was to improve on the two-step system by further integrating the dissolution and permeation components.

As a first objective, a continuous dissolution/Caco-2 system was developed and predicted dissolution-absorption relationships for fast and slow dissolving formulations of piroxicam, metoprolol tartrate, and ranitidine HCl. Like the two-step system, the continuous system qualitatively matched observed dissolution-absorption relationships from clinical studies, and usually matched clinical profiles quantitatively. For example, the system successfully predicted the slow metoprolol formulation and slow ranitidine formulation to be permeation-rate-limited. Also, the system successfully predicted slow piroxicam formulation to be mostly dissolution-rate-limited, and the fast piroxicam formulation to be permeation-rate-limited, in spite of piroxicam's high permeability and low solubility. Hence, this system appears to have utility to identify drug absorption from solid oral dosage forms as either dissolution or permeation-rate-limited, without mathematical model-dependant assumptions.

Given results from ranitidine FAST to enhance permeability in vitro, in a second objective, the disintegrant croscarmellose sodium was identified as the permeation-enhancing agent. Croscarmellose sodium increased ranitidine permeability 10-fold in the dissolution/Caco-2 system in vitro.

In a third objective, solubilizing agents in various piroxicam formulations had a minimal to significant impact on dissolution, while did not influence permeability. While anticipated, these results support the utility of the dissolution/Caco-2 system as a tool to study excipient effects on dissolution and intestinal permeation kinetics. These results

represent negative control studies for the effect of excipient on drug permeation and will help provide a basis to consider a permeation-enhancing effect of an excipient in future applications of the continuous dissolution/Caco-2 system, particularly given the above "false positive" permeation-enhancing effect of croscarmellose.

These results indicate a continuous dissolution/Caco-2 system may have utility in the biopharmaceutical characterization of oral solid dosage form performance, beyond discrete solubility and permeability determinations. However, further evaluation of this system is needed to assess its general applicability and limitations. Further evaluation should include the examination of more drugs of diverse biopharmaceutical properties and their dosage forms. Additionally, evaluation of other, more permeable, intestinal membrane models is encouraged, due to (a) the sensitivity of dissolution-absorption trajectories in the neighborhood of $\alpha = 1$ and (b) Caco-2 monolayer's lower permeability relative to human tissue.

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